

Supplemental Figures and Tables

Supplemental Figure 1

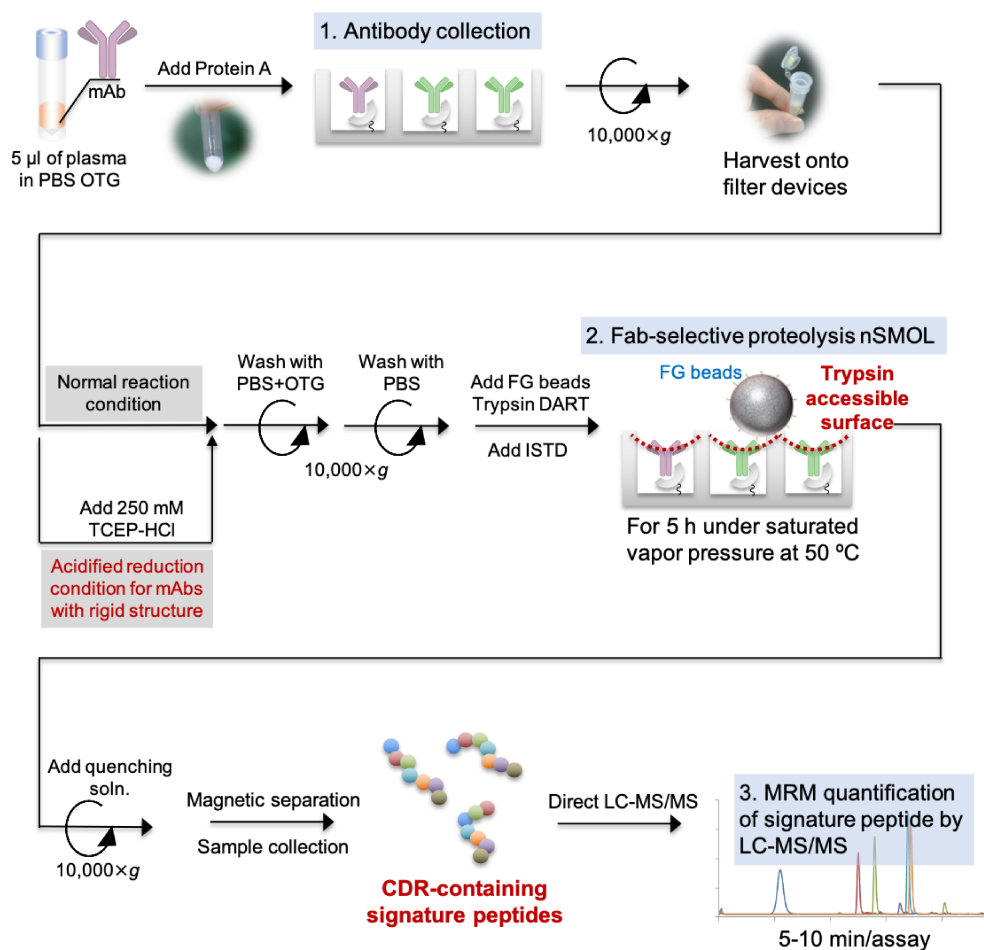


Figure S1. An overview of nSMOL assay.

A workflow of the nSMOL assay mainly consists of three steps: (1) IgG collection from biological samples using Protein A resin, (2) Fab-selective proteolysis using immobilized trypsin nanoparticles, and (3) LC-MS/MS quantitation of signature peptides using MRM analysis.

Figure S2

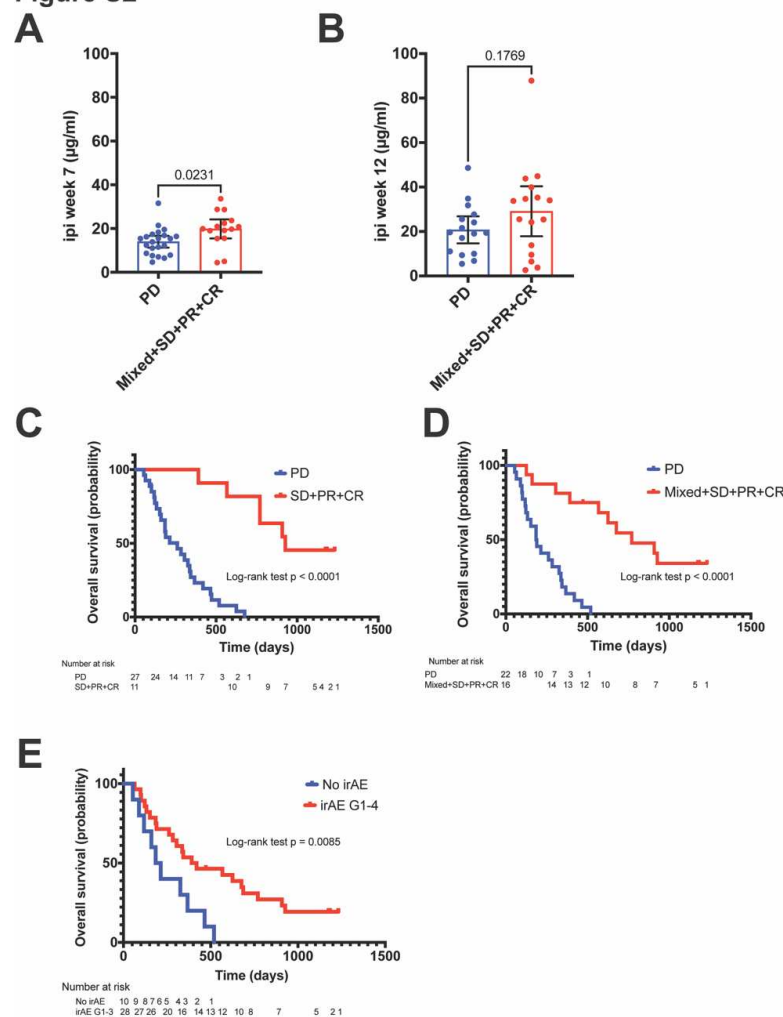


Figure S2. Ipilimumab trough levels based on clinical benefit and OS based on clinical benefit or irAE status. A) The trough levels of ipilimumab at week 7 were compared between patients with disease progression and patients without progression including mixed response (n= 22, n= 15, respectively). B) The trough levels of ipilimumab at week 12 were compared between patients with disease progression and patients without progression including mixed response (n= 16, n= 16, respectively). PD: progressive disease, Mixed: mixed response, SD: stable disease, PR: partial response, and CR: complete response. Curves for OS based on clinical benefit (C), clinical benefit including mixed response (D), or irAE status (E). The survival curves are significantly different by the Log-rank test (C and D: $p < 0.0001$, E: $p = 0.0085$.)

Figure S3

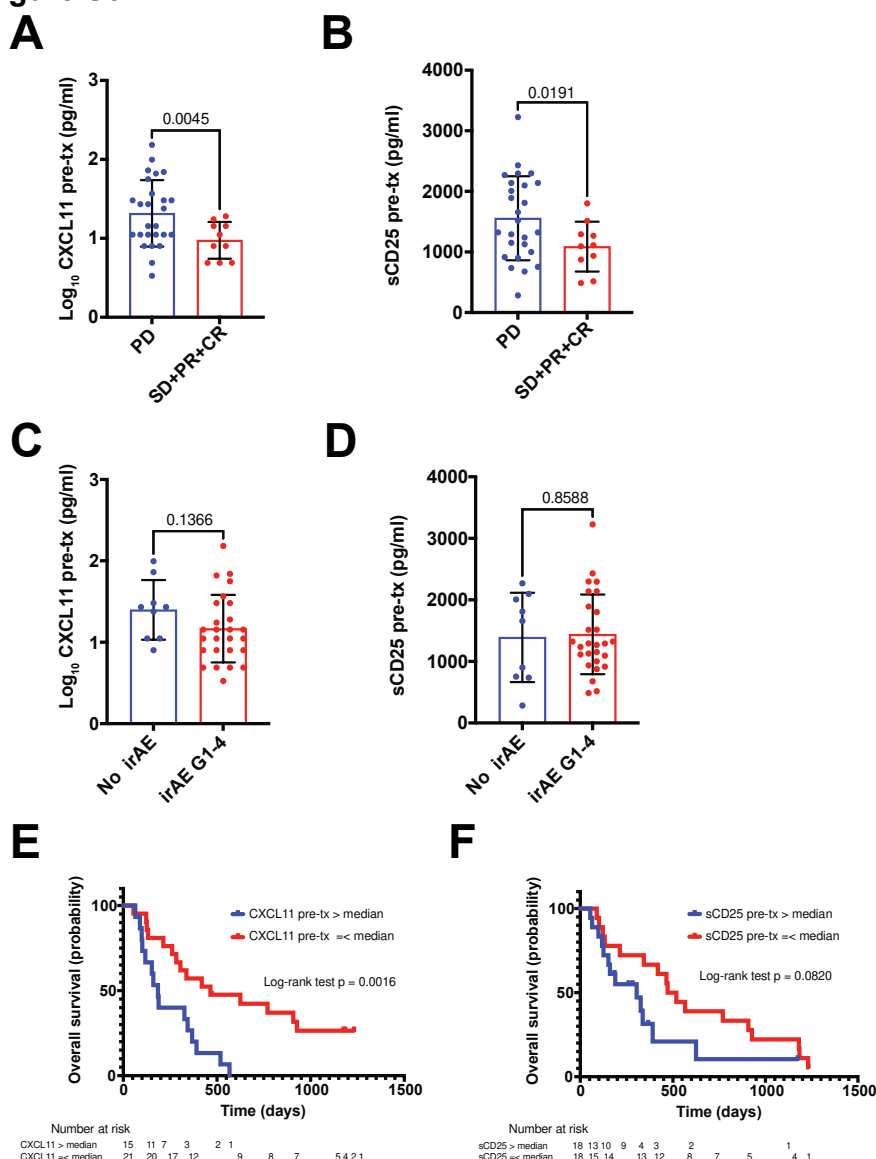


Figure S3. Relationships between pre-treatment CXCL11 or sCD25 levels and clinical data. Pre-treatment levels of CXCL11 (A) or sCD25 (B) were compared between patients with (n=26) or without (n=10) progression. Pre-treatment levels of CXCL11 (C) or sCD25 (D) were compared between patients who did not develop (n=9) and those who developed irAE (n=27). Curves for OS based on pre-treatment CXCL11 levels (E) or sCD25 levels (F) are shown.

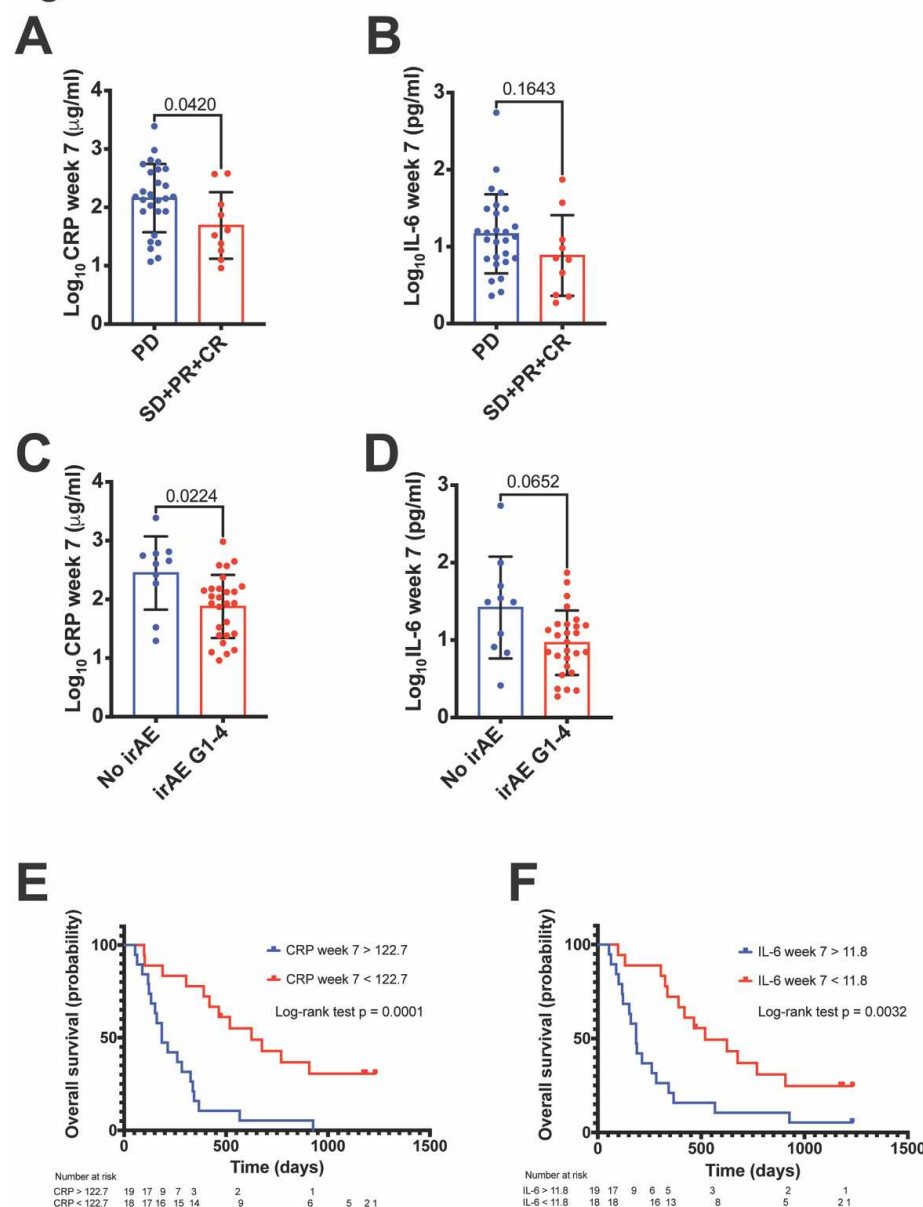
Figure S4

Figure S4. Relationships between on-treatment CRP or IL-6 levels and clinical data. Levels of CRP (A) or IL-6 (B) at week 7 were compared between patients with (n=27) or without (n= 10) progression. Levels of CRP (C) or IL-6 (D) at week 7 were compared between patients who did not develop irAE (n=10) and those who did (n=27). Curves for OS based on CRP levels (E) or IL-6 levels (F) at week 7 are shown.

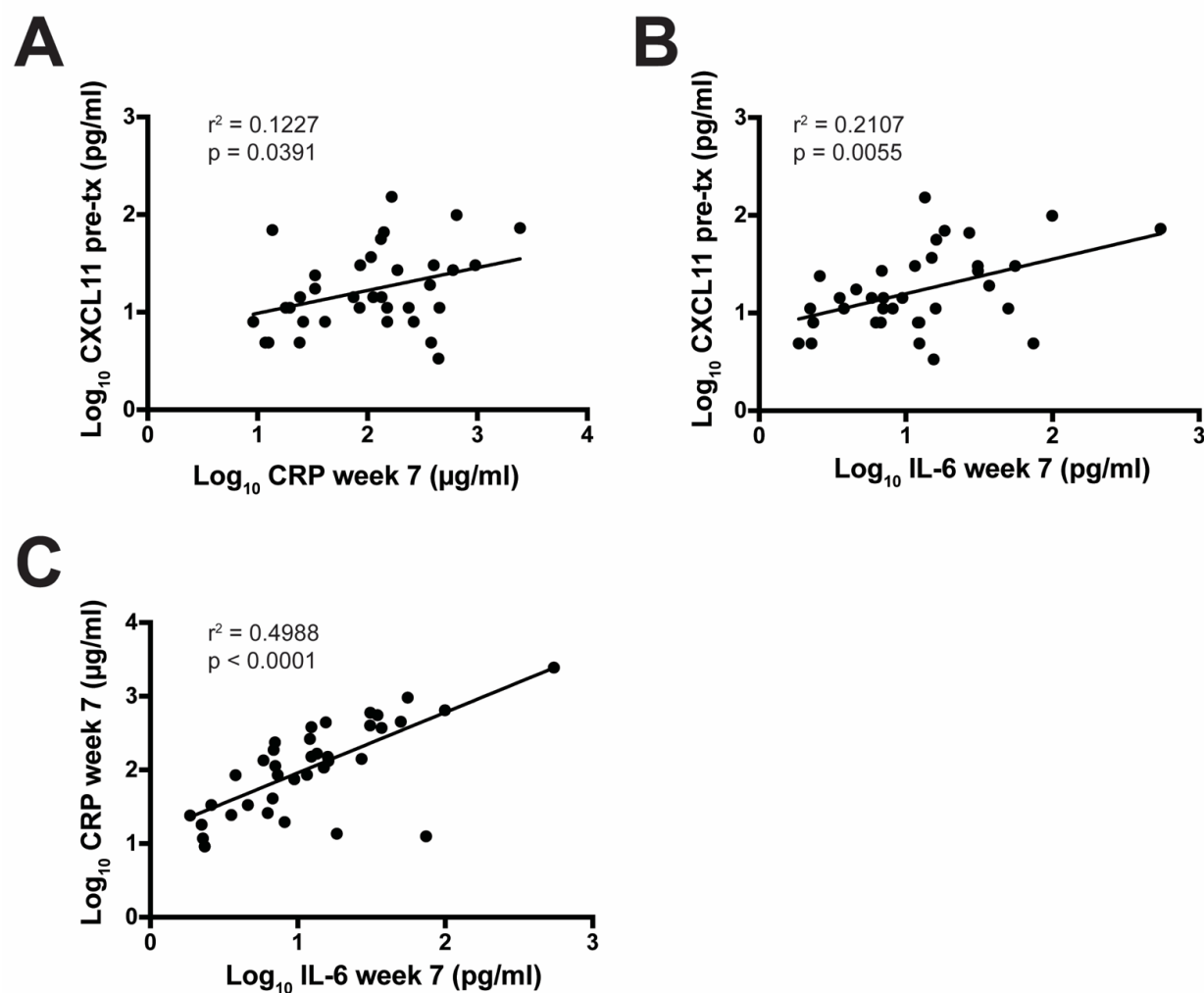
Figure S5

Figure S5. Relationship among pre-treatment levels of CXCL11, levels of CRP at week 7, and levels of IL-6 at week 7. The relationships between pre-treatment levels of CXCL11 and levels of CRP at week 7 (A), between pre-treatment levels of CXCL11 and levels of IL-6 at week 7 (B), or between levels of CRP at week 7 and levels of IL-6 at week 7 (C) are shown.

Table S1. Demographic information (n=38)

		N (%)
Age	Median (years; range)	58 (36-81)
Sex	Male	24 (63)
	Female	14 (37)
ECOG PS	0	13 (34)
	1	23 (61)
	2	2 (5)
BMI	Male Median (range)	26.7 (20.6-34.5)
	Female Median (range)	25.9 (19.4-29.3)
Melanoma diagnosis	Cutaneous	33 (87)
	Mucosal	2 (5)
	Uveal	3 (8)
Disease Stage	III	1 (3)
	IV	37 (97)
Clinical response	PD	28 (74)
	SD	1 (3)
	PR	7 (18)
	CR	2 (5)
Survival	Median (months; range)	11.3 (1.8-41.1)
irAE*	No	10 (26)
	Grade 1	19 (50)
	Grade 2	7 (18)
	Grade 3	1 (3)
	Grade 4	1 (3)
ipilimumab dose	3 mg/kg	38 (100)
	10 mg/kg	0 (0)
Prior immunotherapy	No	23 (61)
	Yes	15 (39)

*When the subject developed multiple irAEs, the subject was assigned to the group based on the highest irAE grade.

Table S2. Validated MRM transition for the ipilimumab signature peptide

Selected peptide	Location	Optimal MRM condition				Role
		Transition mass filter [m/z]	Q1 [V]	Collision [V]	Q3 [V]	
TGWLGPFDYWGQGT VTVSSASTK	H-CDR3	853.5→780.4 (y8 ⁺)		-29	-38	Quantitation
		853.5→580.3 (y6 ⁺)	-38	-26	-38	Structure
		853.5→345.2 (b3 ⁺)		-31	-23	Structure

Table S3. The summary of the precision and accuracy of ipilimumab

TGWLGPFDYWGQGTLVTVSSASTK

Assay	Theoretical concentration	Set concentration (µg/mL)			
		1.0	2.5	40.0	100
Average (N=15)	Mean	1.04	2.45	39.5	101
	SD	0.0980	0.148	3.46	6.47
	CV (%)	9.46	6.03	8.77	6.41
	Accuracy (%)	104	97.9	98.8	101